Available online at www.sciencedirect.com
ScienceDirect
Postharvest Biology and Technology 48 (2008) 341–346
Postharvest Biology and Technology
www.elsevier.com/locate/postharvbio

Non-destructive freeze damage detection in oranges using machine vision and ultraviolet fluorescence

D.C. Slaughter [a,*], D.M. Obenland [b], J.F. Thompson [a], M.L. Arpaia [c], D.A. Margosan [b]

[a] *Biological and Agricultural Engineering, University of California, Davis, One Shields Avenue, Davis, CA 95616, United States*
[b] *Agricultural Research Service, U.S. Department of Agriculture, 9611 South Riverbend Avenue, Parlier, CA 93648, United States*
[c] *Botany & Plant Sciences, University of California, Riverside, CA 92521, United States*

Received 9 July 2007; accepted 1 September 2007

Abstract

A non-contact, non-destructive, and rapid method of detecting freeze-damaged oranges based on ultraviolet (UV) fluorescence of the peel oil constituents visible on the peel surface was investigated. The visual appearance is different from oleocellosis in that freeze-damaged oranges exhibit a fine pattern of 1–2 mm bright yellow dots on the peel when viewed under longwave UV light. Visual and machine vision-based methods were evaluated to determine their ability to detect freeze damage in Californian navel oranges (*Citrus sinensis* L. Osbeck) subjected to laboratory simulated freeze conditions of −7 °C for 0, 8, or 16 h periods. The study focused on the period within the first few days (i.e., prior to fruit dehydration) after a freeze event has occurred because there are currently no rapid, objective, and non-destructive methods of freeze damage detection available for use during that time period. Using the USDA segment cut method to determine the presence of internal freeze damage, the classification rates for both UV fluorescence methods varied with the level of freeze damage. Using machine vision, a classification accuracy of 87.9% was obtained for unfrozen and moderately or severely frozen fruit, dropping to 64.4% for fruit with low levels of freeze damage. UV fluorescence shows promise for both visual inspection using existing black light inspection rooms or for automation using on-line machine vision techniques for separating freeze-damaged fruit subjected to moderate or severe freeze conditions.

Keywords: Citrus; Ultraviolet; Fluorescence; Non-destructive; Freeze damage; Machine vision

1. Introduction

Oranges subjected to freezing conditions are frequently unsuitable for consumption because they have off-flavors or dehydrated flesh. Intracellular ice formation damages the cells in frozen oranges creating pathways for moisture loss that can result in the dehydration of freeze-damaged fruit (Syvertsen, 1982). Freezing can also cause adverse chemical changes that result in volatile production and under some conditions, the formation of limonin, which causes the fruit to have a bitter taste (Sinclair, 1984). California Department of Food and Agriculture regulations do not permit oranges to be sold if more than 15% of fruit in a lot have scorable freeze damage (USDA, 1999). There are currently no automated or rapid methods for detecting freeze-damaged oranges within the first few weeks (i.e. prior to fruit dehydration) after a freeze event has occurred. Currently, during the first few weeks after a freeze event, damage is assessed by inspectors who look for damaged flesh in the fruit using the segment cut method (USDA, 1999). This procedure is slow and subjective and the California citrus industry would benefit from the development of a rapid instrumental method of detecting freeze-damaged oranges.

The only non-destructive method commonly used in California to separate freeze-damaged from sound fruit is by density separation using flotation, Hatton and Cubbedge (1978), or more recently using machine vision and weight sensors, Miller et al. (2006). However, this method cannot be used until sufficient moisture loss has occurred in the freeze-damaged fruit, which typically requires a delay of a few weeks time after the freeze event. Packinghouse operators also attempt to remove freeze-damaged fruit by sorting out fruit with sunburn marking on the peel because fruit with this damage is located on the outer portion of the tree canopy and is most susceptible to freezing.

Several researchers have reported laboratory investigations of non-destructive methods for freeze-damage detection in citrus that might be used shortly after a freeze event. Using gas

* Corresponding author. Tel.: +1 530 752 0102; fax: +1 530 752 2640.
E-mail address: dcslaughter@ucdavis.edu (D.C. Slaughter).

0925-5214/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.postharvbio.2007.09.012

chromatograph–mass spectrometer methods, Obenland et al. (2003) non-destructively measured the headspace volatile emissions of intact navel oranges, with 10 oranges sealed in a glass jar for an hour, and found that the emission of ethanol, ethyl butanoate, methyl hexanoate and ethyl octanoate in navel oranges were strongly enhanced by freezing. No enhancement in volatile emission induced by chilling the fruit was observed unless the treatment resulted in intracellular ice formation. In a similar study, Tan et al. (2005) used a hand-held ethanol sensor (sensitive to 0.03 mg/L ethanol) to measure the headspace ethanol levels of individual Valencia oranges stored 1 h in glass jars. They observed no ethanol production in unfrozen oranges, and detected ethanol in the headspace of 37% of freeze-damaged fruit using this sensor. In subsequent studies, Thompson and Slaughter (2005) and Thompson et al. (2006) used a more sensitive version (sensitive to 0.01 mg/L ethanol) of the hand-held ethanol sensor of Tan et al. (2005) to measure the headspace ethanol levels of navel oranges held in one-quart sealed plastic bags for 1-h. Taking advantage of the lack of ethanol detected in unfrozen oranges by Tan et al., they analyzed the fruit in small batches of 6 or 7 fruit, where a batch of fruit was consider frozen if any of the fruit in the batch had headspace ethanol levels >0.01 mg/L. Comparing these results to the USDA segment cut method for the batch showed classification accuracies ranging from 78% to 100% in laboratory studies of 'Atwood' and 'Washington' navel oranges harvested in the 2005 and 2006 seasons. Gambhir et al. (2005) measured the proton spin–spin relaxation times (T2) of navel orange peel and flesh segments using a nuclear magnetic resonance (NMR) spectrometer. Exposure to freezing (−7 °C) temperatures for 20 h did not affect the T2 values of the peel, but freezing caused a 15% decrease in the T2 values of navel orange flesh segments. While NMR is potentially a non-destructive method additional study is required to determine if it can be used for detection of intact freeze-damaged oranges.

Recently, Obenland and Margosan (unpublished data) discovered that freeze-damaged oranges, grapefruit and tangerine exhibit a pattern of 1–2 mm bright yellow dots on the peel when viewed under longwave UV light (365 nm), (Fig. 1). The fluorescent pattern is likely due to the fluorescence of tangeritin, a polymethoxylated flavone (Swift, 1967; Dugo et al., 2005), in the peel oil of these fruits, Figs. 2 and 3. The fluorescent pattern becomes visible within 2 h of thawing in the peel of intact fruit when oil glands in the peel rupture during a freeze event allowing the oil to diffuse through the peel toward the peel surface.

The freeze damage peel oil phenomena is different from oleocellosis, a physiological peel disorder in citrus caused by mechanical damage to the peel (Fawcett, 1916; Cahoon et al., 1964; Knight et al., 2002). Oleocellosis results in surface blemishes that are visible when illuminated by visible light, while the peel of freeze-damaged fruits cannot be visually distinguished from that of undamaged fruit under normal viewing conditions unless the damage is very severe. The fluorescent pattern due to freeze damage can be distinguished from mechanical, or mold related, or other types of damage resulting in oil gland rupture by the visual texture of the pattern. For example, mechanical damage due to rough handling at harvest typically appears as large (greater than 5 mm in size) consolidated blemishes on the peel rather than the fine pattern of very small dots shown in Fig. 1. Visual inspection of citrus under UV illumination (i.e. in "black light" rooms) is commonly used in Californian citrus packinghouses to remove fruit subject with fungal infection. The method has not been evaluated for removal of freeze-damaged fruit.

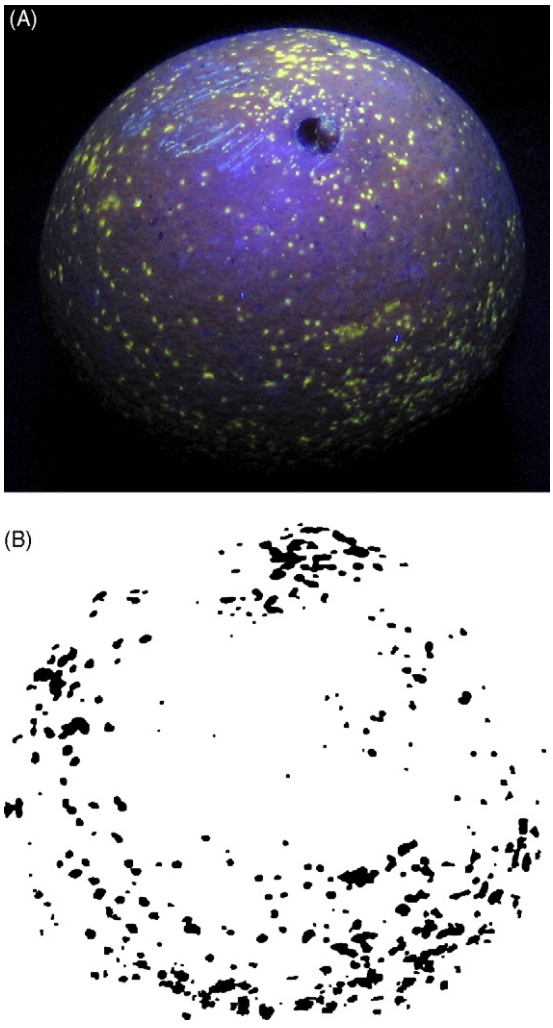

Fig. 1. (A) Yellow spot pattern visible in freeze-damaged oranges a few hours after thawing when viewed under 365 nm illumination. (B) Automatically segmented image of orange in part a showing yellow spot extraction. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

The objective of this study was to evaluate the feasibility of using longwave UV fluorescence to detect freeze-damaged oranges and to compare this method with the standard USDA segment cut method of identifying freeze-damaged oranges. Because a natural freeze is an uncommon event, simulated freeze conditions in the laboratory were used for this experiment.

2. Materials and methods

Californian navel oranges, (*Citrus sinensis* L. Osbeck), grown at the University of California Lindcove Research and Education Center, Exeter, CA, were harvested on a weekly basis

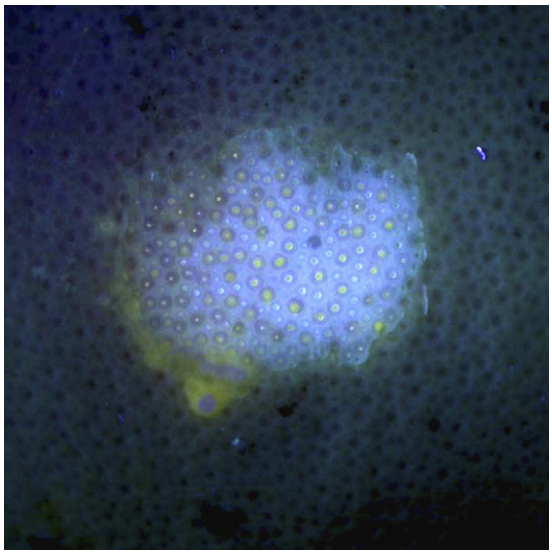

Fig. 2. Yellow fluorescence emission in the oil glands of an unfrozen grapefruit visible after a thin layer of the rind has been removed and illuminated by 365 nm light. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

over a 6-week period from mid-January through the end of February 2006, shipped to a laboratory at the UC Davis campus and stored in a low-temperature incubator (Model 307C; Fisher Scientific, Pittsburgh, PA) at 5 °C for less than 72 h before testing. The oranges were handpicked and were not chemically treated postharvest. Only sound unfrozen fruit of about 10 cm in diameter, with no visible physical defects, were used in the study.

Field freezing of citrus fruit was simulated in the laboratory using a modified household chest freezer (Model Kenmore 253; Sears Roebuck & Co., Chicago, IL) set to a constant −7 °C temperature using the control system described by Tan et al. (2005). About 81 fruit from each harvest (for a total of 493 fruit) were randomly split into three groups and each group subjected to one of three temperature treatments: chilled at 5 °C for 16 h, chilled at 5 °C for 8 h and then placed in the freezer for 8 h, and placed in the freezer for 16 h. These treatments were designed

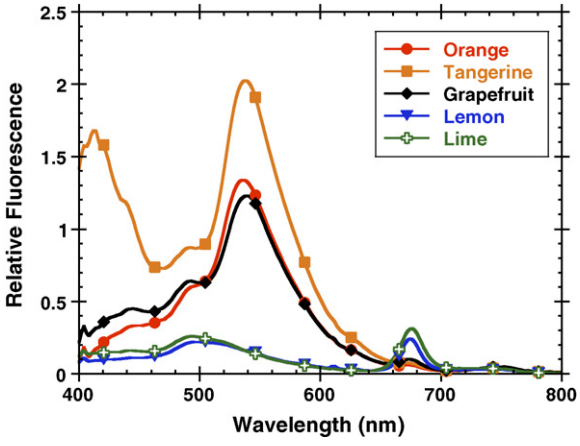

Fig. 3. Fluorescence spectra of the peel oil of five types of citrus when illuminated with 365 nm light.

to simulate an unfrozen control, moderate, and severe freezing conditions and to provide fruit with a range of freeze damage. After each treatment, the fruit were placed at 21 °C overnight (about 15 h) to allow the fruit to thaw.

After thawing, the level of freeze damage in each fruit due to the applied temperature treatment was visually evaluated under UV illumination (365 nm light, Blak-ray, model B-100AP, UVP Upland, CA) using a 10-point scale ranging from 0 (no peel damage), to 5 (complete peel damage) in 0.5 unit increments, Fig. 4. After visual assessment, color digital images (Canon, camera model A95, 0.085 mm/pixel resolution) of the top and bottom of each fruit were collected. The fruit were illuminated with the same 365 nm light source during image acquisition. The fruit were then evaluated for freeze damage using the official segment cut method of the US Department of Agriculture (USDA, 1999) by two individuals who worked together as a team to judge each fruit. The individuals performing the segment cut method were trained by experienced inspectors from the California Department of Food and Agriculture. The inspectors were not aware of the type of temperature treatment that a fruit was subjected to at the time of inspection.

The yellow spot pattern associated with the UV fluorescence of freeze-damaged peel tissue had a unique and high contrast color compared to undamaged peel tissue that allowed simple and high-speed image processing techniques to be used in quantifying the level of damage. The digital color images of each fruit were automatically evaluated (using a macro script in the ImageJ software package, NIH, 2006) to determine the percentage of the peel surface covered by yellow fluorescent spots due to freeze damage using the following image processing steps. The image resolution was reduced from 0.085 to 0.33 mm/pixel to reduce image processing time requirements. The total area of yellow spots in the image was determined by transforming the image into the hue, saturation, and intensity color space and then segmenting the hue image for gray levels between 35 and 100, Fig. 1B. The total area of the fruit in the image was determined by segmenting the green image of the red, green, blue color image with a gray level threshold above 20. The ratio of spot area to fruit area in the image was then determined and expressed as a percentage.

The ability of the visual peel damage score and the automatic machine vision yellow spot percentage measurement to predict USDA segment cut freeze damage classes were determined using a Bayesian classifier and discriminant analysis (SAS Proc Discrim, 2004). Classification performance levels were then determined for each temperature treatment.

3. Results and discussion

One percent of the unfrozen oranges, 49% of the 8-h freeze treated, and 78% of the 16-h freeze treated oranges were classified as frozen according to the USDA segment cut method, Table 1. The segment cut method is subjective and its ability to accurately judge the marketability of the fruit has not been fully evaluated. In particular fruit taste, which is a critical factor in determining the consumer acceptability of the fruit, has not

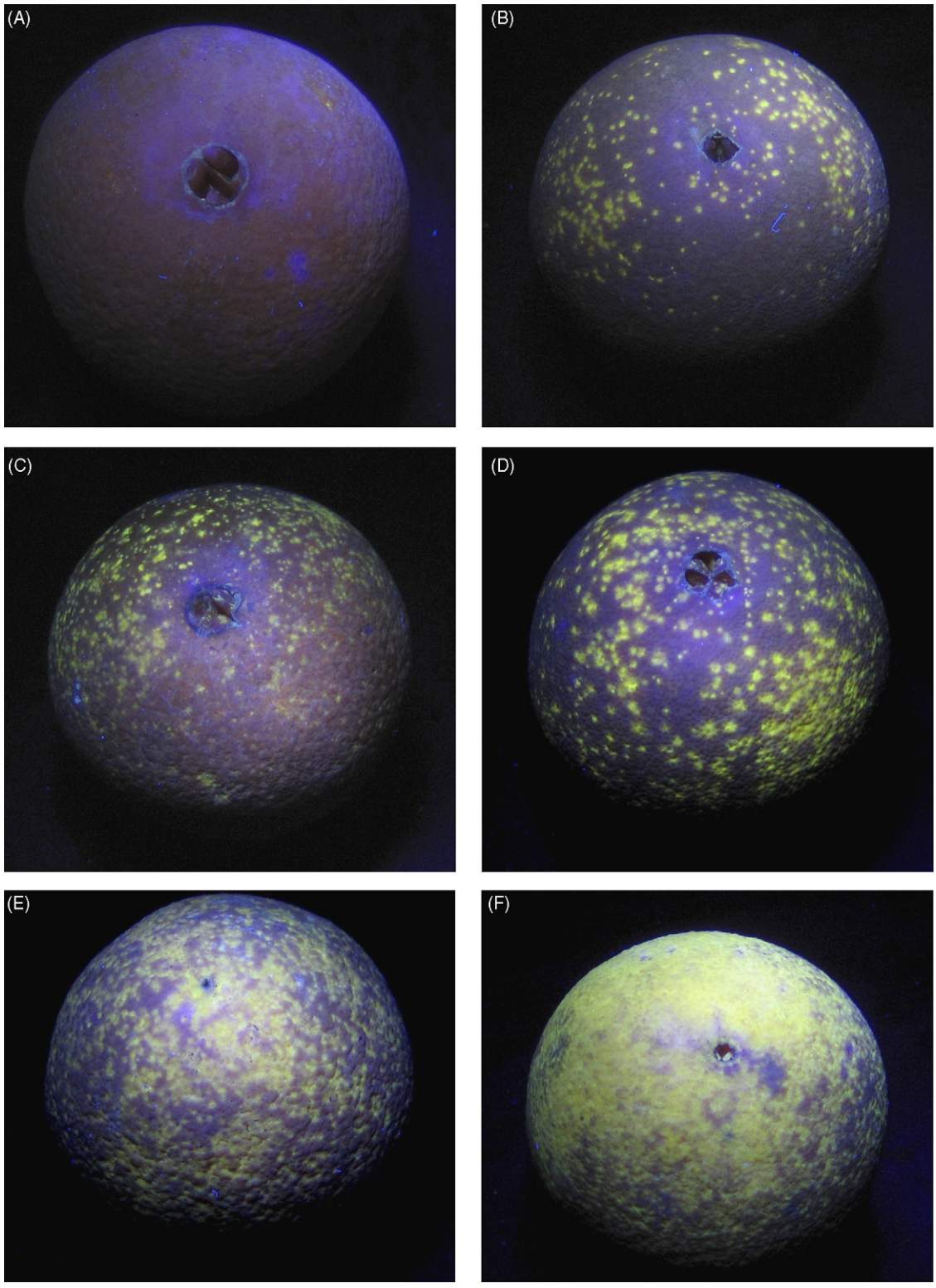

Fig. 4. Photographs of freeze-damaged oranges showing the visual scores used to define the level of damage observed for the freeze treatments applied in the study. (A) 0 = no damage, (B) 1 = slight damage, (C) 2 = noticeable damage, (D) 3 = moderate damage, (E) 4 = severe damage, and (F) 5 = extreme damage. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

been evaluated in conjunction with the USDA method. Informal observations in this study indicated that the segment cut method was not a consistent predictor of off-flavor in fruit classified as frozen by the method.

The average and standard deviation values of the visual (UV fluorescence) peel damage score and the machine vision yellow spot percentage for each of the three temperature treatments are shown in Table 1. The average values increase with the severity

Table 1
Average and standard deviation levels of frozen fruit, visual (UV fluorescence) peel damage score, and machine vision spot percentage for three temperature treatments

Treatment	*N*	USDA frozen (%)	Visual damage score (mean/standard deviation)[a]	Machine vision spot percentage[a] (mean/standard deviation)
Chilled	168	1	0.22 a/0.45	0.42% a/0.84%
8-h freeze	166	49	0.90 b/0.84	4.43% b/6.44%
16-h freeze	159	78	2.06 c/1.61	15.16% c/17.09%

[a] Mean values with the same grouping letter are not significantly different at the $\alpha = 0.05$ level. The harmonic mean sample size was used in the analysis due to unequal sample numbers for each treatment.

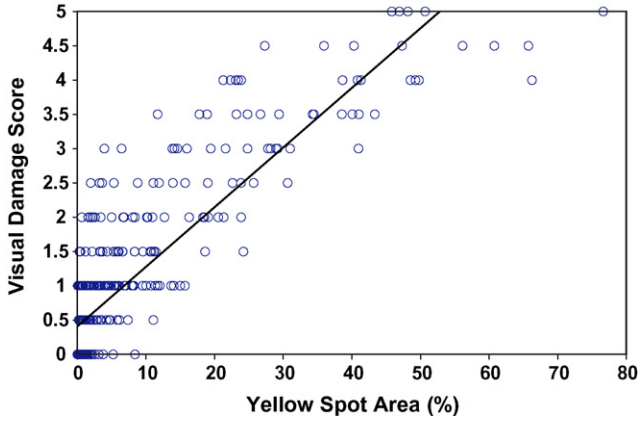

Fig. 5. Relationship between the visual damage score and the yellow spot area determined by machine vision. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

of the freeze treatment and parallel the increase in the number of fruit that are scorable for freeze-damage using the segment cut method. The relationship between the yellow spot percentage determined by machine vision and the visual damage score is shown in Fig. 5. The relationship is quite linear and the two measures are correlated ($r^2 = 0.74$). The linear regression model for this data is:

$$\text{visual damage score} = 0.41 + 0.087 \times \text{yellow spot area by machine vision} \quad (1)$$

Discriminant analysis was used to evaluate the classification potential of both the visual damage score and the machine vision spot area measurement for classifying fruit as frozen or unfrozen by the segment cut method. The classification table for the visual damage score, Table 2, shows that 70.1% of the unfrozen fruit and 70.3% of the frozen fruit were classified correctly for a total error rate of 29.8%. The classification equation, determined by discriminant analysis, for the visual damage score was:

$$\text{If visual damage score} \leq 0.5 \text{ then unfrozen, else frozen} \quad (2)$$

Table 2
Freeze damage classification table

Classification by segment cut method	Visual damage score method, classification accuracy (%)	Machine vision method, classification accuracy (%)
USDA unfrozen	70.1	80.4
USDA frozen	70.3	68.3

The classification table for the machine vision spot area measurement, Table 2, shows that 80.4% of the unfrozen fruit and 68.6% of the frozen fruit were classified correctly for a total error rate of 28.3%. The classification equation, determined by discriminant analysis, for the machine vision spot area measurement was:

$$\text{If yellow spot area} < 2\% \text{ then unfrozen, else frozen} \quad (3)$$

Although both methods have comparable error rates, the visual method had balanced classification rates between frozen and unfrozen categories while the machine vision method did a better job of identifying unfrozen fruit at the cost of a small reduction in the ability to detect frozen fruit. In addition, the error rate was not uniformly distributed across UV fluorescence levels. For example, of the 46 fruit with a visual damage score of 0.5, only 30% were correctly classified, while 83% of the 196 fruit with a visual damage score of 0 were correctly classified. The average classification rate for oranges with either a visual damage score of 0 or a score greater than 2.5 was 86.4%. A similar pattern occurred for the machine vision method, where fruit with yellow spot area of 0% or with an area greater than 11% had an average classification rate of 87.9% while those between 0% and 11% had a classification rate of 64.4%. This indicates that the method is better suited for classifying fruit with moderate to severe damage levels than fruit with low levels of damage. We believe that the two main sources of error were the subjective nature of the segment cut method and the fact that the UV fluorescence method is a measure of peel damage and not a direct measure of flesh damage.

While these classification rates are lower than desired, these two classification systems can provide value to a grower in situations where a lot fails the CDFA 15% limit for freeze-damaged fruit. Without a non-destructive method for identifying and removing freeze-damaged fruit, the entire lot would not be saleable on the fresh market. In many cases the overall quality of the lot may be improved to meet the CDFA limit for freeze damage by employing a classifier with a total error rate less than 50%. While it is true that a classifier with a non-zero error rate will incorrectly discard good fruit, the net effect may be financially advantageous to the grower if the sorted lot can meet the CDFA limitation on freeze-damaged fruit and command fresh market prices. The value of a specific classifier is a function of the error rate distribution across damage levels and the amount of freeze-damaged fruit in the lot.

The total classification rates observed for the UV fluorescence methods were similar to those found by Tan et al. (2005) using an ethanol headspace sensor for freeze detection. The

main difference was that the ethanol sensor was 100% accurate for unfrozen fruit but only 37% accurate for frozen fruit. While both of these techniques are non-destructive, the UV fluorescence method is suited for real-time inspection of all fruit on citrus packing lines whereas the ethanol sensing method is only suited for evaluating small batches of fruit (e.g. 7–10 fruit in sealed jars or plastic bags) due to the 100% accuracy on unfrozen fruit and the 1 h delay to allow the headspace ethanol to stabilize.

Another potential advantage to the UV fluorescence technique is that it could be implemented fairly quickly by human inspectors in existing black light inspection rooms in packing lines, requiring only a small amount of additional training and no additional capital expenditures. Currently visual inspection is done in black light rooms to remove fruit with fungal infection. Training inspectors to also identify fruit with small yellow fluorescent spots should be feasible.

4. Conclusions

The small dot pattern visible on the peel of oranges when illuminated by longwave UV light (365 nm) can be used to identify fruit with moderate to severe levels of freeze damage. The method is suitable for both visual inspection using black light inspection rooms or for automation using machine vision techniques. When compared to the existing USDA segment cut method of freeze damage evaluation, the UV fluorescence method had classification overall accuracies of about 70% and the accuracy increased to 86% for fruit with no UV fluorescence or for those fruit with moderate to severe levels of freeze damage. Additional research is required to fully evaluate the relationship between peel damage and flesh damage in citrus under naturally occurring freeze events.

Acknowledgements

The authors would like to thank the California Citrus Research Board for partially funding this project and the Lindcove Research and Education Center for providing the oranges used in this study.

References

Cahoon, G.A., Grover, B.L., Eaks, I.L., 1964. Cause and control of oleocellosis on lemons. Proc. Am. Soc. Hort. Sci. 84, 188–198.

Dugo, P., Mondello, L., Favoino, O., Cicero, L., Rodriguez Zenteno, N.A., Dugo, G., 2005. Characterization of cold-pressed Mexican Dancy tangerine oils. Flavour Fragr. J. 20, 60–66.

Fawcett, H.S., 1916. A spotting of citrus fruits due to the action of oil liberated from the rind. Calif. Agric. Exp. Station Bull. 266, 259–270.

Gambhir, P.N., Choi, Y.J., Slaughter, D.C., Thompson, J.F., McCarthy, M.J., 2005. Proton spin–spin relaxation time of peel and flesh of navel orange varieties exposed to freezing temperature. J. Sci. Food Agric. 85, 2482–2486.

Hatton, T.T., Cubbedge, R.H., 1978. Separation of frozen grapefruit by using emulsions of differing specific gravities. Proc. Fla. State Hortic. Soc. 91, 126–128.

Knight, T.G., Klieber, A., Sedgley, M., 2002. Structural basis of the rind disorder oleocellosis in Washington navel orange (*Citrus sinensis* L. Osbeck). Ann. Bot. 90, 765–773.

Miller, W.M., Wardowski, W.F., Grierson, W., 2006. Separation and grading of freeze-damaged fruit. In: Wardowski, W.F., Miller, W.M., Hall, D.J., Grierson, W. (Eds.), Fresh Citrus Fruits. Florida Science Source, Inc., Longboat Key, FL, pp. 299–306.

NIH ImageJ, 2006. Image processing and analysis in Java. http://rsb.info.nih.gov/ij/.

Obenland, D.M., Aung, L.H., Bridges, D.L., Mackey, B.E., 2003. Volatile emissions of navel oranges as predictors of freeze damage. J. Agric. Food Chem. 51, 3367–3371.

SAS, 2004. SAS/Stat Statistical Analysis Software, v. 9.1. SAS Institute, Inc., Cary, NC.

Sinclair, W.B., 1984. The Biochemistry and Physiology of the Lemon and Other Citrus Fruits. University of California, Division of Agriculture and National Resources, Oakland, CA.

Swift, L.J., 1967. TLC-spectrophotometric analysis for neutral fraction flavones in orange peel juice. J. Agric. Food Chem. 15, 99–101.

Syvertsen, J.P., 1982. Dehydration of freeze-damaged oranges. HortScience 17, 803–804.

Tan, E.S., Slaughter, D.C., Thompson, J.F., 2005. Freeze damage detection in oranges using gas sensors. Postharvest Biol. Technol. 35, 177–182.

Thompson, J.F., Slaughter, D.C., 2005. Survey of Sensing Methods for Detection of Freeze Damage—Annual Progress Report for Project CRB No. 5600-17. California Citrus Research Board, Visalia, CA.

Thompson, J.F., Slaughter, D.C., McCarthy, M.J., 2006. Survey of Sensing Methods for Detection of Freeze Damage—Annual Progress Report for Project CRB No. 5600-17. California Citrus Research Board, Visalia, CA.

USDA, 1999. Arizona California Citrus Loss Adjustment Standards Handbook. FCIC-25040 (11-1999). USDA, Washington, DC.